CHARACTERIZATION OF DNA IN WHEAT CHLOROPLASTS ISOLATED BY A NEW "LACERATION TECHNIQUE"

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1. Introduction

DNA has been well characterized in chloroplasts from lower plants and dicotyledons, but not in monocotyledons. Generally, the demonstration of chloroplast DNA is made by CsCl sedimentation of a DNA preparation containing both nuclear and chloroplast DNA. Unequivocal proof that nuclear type DNA is not present in the chloroplasts can be obtained by isolating chloroplasts free from nuclear contamination. This paper reports on the demonstration of DNA in chloroplasts from leaves of wheat, a monocotyledonous plant and describes a simple "laceration technique" for isolating whole chloroplasts free from nuclear contamination. In another method of isolation of chloroplasts it was impossible to separate chloroplast DNA from nuclear DNA by MAK column chromatography, although this could be achieved by CsCl sedimentation. The buoyant densities of nuclear and chloroplast DNA were 1.702 ± 1 and 1.714 ± 1 respectively.

2. Materials and methods

2.1. *Chloroplast preparations*

2.1.1. Modified procedure of Jensen and Bassham [1]

About 20 g ten day old primary leaves of greenhouse grown wheat (*Triticum aestivum L.* var Selkirk) were washed, chilled and ground for 10 sec in a Waring blendor with 80 ml of solution A [1]. The suspension was filtered through four layers of cheesecloth and centrifuged for 50 sec at 2,000 × *g*. The pellet containing chloroplasts was resuspended in solution B [1] and centrifuged in an IEC rotor SB206 for 2 hr at 30,000 × *g* on a preformed gradient of sucrose (20–60% in solution B). Whole nuclei and some starch grains were completely sedimented. All operations were conducted at 3°.

2.1.2. Laceration technique

About 10 g of wheat leaves were placed in a glass solvent trough (34 cm in length) containing 25 ml of solution A. The leaves were gently lacerated with five small closely spaced scalpels (Cat No 412, German Surgicals, Irex, Toronto) attached to a rubber stopper. The organelles tumble out of the cells into the medium. The suspension was filtered through one layer of Kleenex tissue and the filtrate was centrifuged for 50 sec at 2,000 × *g*. The pellet was suspended in solution B and centrifuged for 20 min at 12,000 × *g* on a sucrose gradient (IEC rotor SB405). The chloroplasts formed a single discrete band.

2.2. *Extraction of DNA*

2.2.1. Method I

The procedure described by Chiang and Sueoka [2] was followed except that (a) the chloroplasts were heated for 10 min at 65° after treatment with 2.5% Sarkosyl N97, (b) Pronase was previously heated for 10 min at 80° to inactivate DNase prior to deproteinization, and (c) after the final dialysis the solution was shaken with an equal volume of chloroform-isoamyl alcohol (24 : 1). The aqueous layer containing DNA was concentrated under a stream of N_2.

2.2.2. Method II

The procedure was the same except that the Pronase step was omitted and proteins were removed by several shakings with chloroform-isoamyl alcohol. The concentrated DNA solution was dialysed against

SSC (NaCl 0.15 M + Na Citrate 0.015 M) for 4 hours. This method was more suitable for MAK chromatography.

2.3. *Density gradient centrifugation of DNA*

DNA was centrifuged in a CsCl gradient to equilibrium using an IEC SB405 rotor for 64 hr at 25° and 35,000 rpm. Fractions collected with a microsiphon were assayed at 260 mμ. For analytical centrifugations the procedure developed by Meselson et al. [3] was used with *M. lysodeikticus* as reference. Buoyant densities are expressed relative to the density of DNA from *E. coli* taken as 1.710, and were calculated from enlarged UV-absorption photographs.

2.4. *MAK column chromatography*

The method of Sueoka and Cheng [4] was followed using stepwise gradient elution with NaCl in 0.05 M phosphate buffer pH 7.2.

3. Results

3.1. *Analysis of DNA after MAK chromatography*

DNA prepared by Method II from chloroplasts isolated by the modified procedure of Jensen and Bassham [3] was chromatographed on a MAK column. Fig. 1 shows that the DNA was eluted in two main peaks at 0.6 M and 0.67 M NaCl. The DNA appearing at 0.62 M and 0.70 M NaCl was due to "tailing" of the two main peaks. Other experiments with enriched nuclei preparations gave a major peak at 0.67 M, but always with a minor peak at 0.60 M NaCl. When DNA eluted at 0.60 M and 0.67 M NaCl was centrifuged to equilibrium in a CsCl gradient the latter gave a symmetrical peak (fig. 2b) whereas the former gave a peak with a shoulder indicating a mixture of nuclear and chloroplast DNA (fig. 2a).

3.2. *DNA from chloroplasts (Jensen and Bassham method)*

DNA was extracted by Method I from chloroplasts isolated by Method Ia. Fig. 3a shows the profile after centrifugation to equilibrium in a CsCl gradient. Two peaks are evident. It is evident that the heavier DNA shown in fig. 3a comes from the chloroplasts since analytical sedimentation of DNA from whole leaves (fig. 4a) has a major peak at 1.702 buoyant density whereas chloroplasts isolated by this method gave a significant peak at density 1.714 and another at 1.702 (fig. 4b). The latter must be due to nuclear contamination since the buoyant density of DNA extracted from enriched nuclei preparations was 1.702 ± 1.

3.3. *DNA from chloroplasts ('laceration technique')*

Microscopic observations under phase contrast of chloroplasts isolated by this technique indicated that there was no fragmentation with no loss of the outer membrane either before or after sucrose density centrifugation. Another advantage of the technique which is especially suitable for leaves with parallel venation

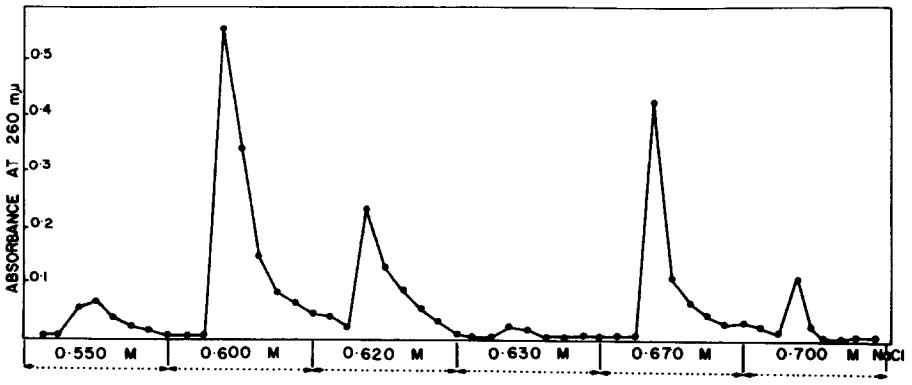

Fig. 1. MAK column chromatography of DNA (Method II) from chloroplasts Method 1a. Stepwise elution with NaCl in 0.05 M phosphate buffer, pH 7.2.

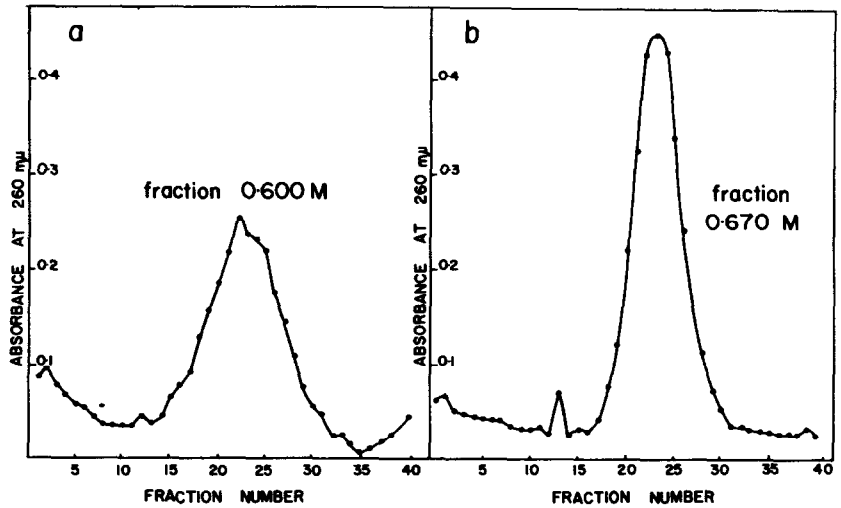

Fig. 2. Sedimentation profile of DNA in CsCl density gradient after chromatography on MAK column. (a) DNA eluted at 0.60 M (fig. 1). (b) DNA eluted at 0.67 M (fig. 1).

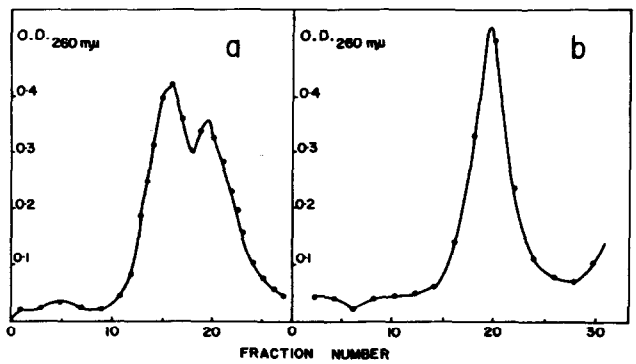

Fig. 3. Sedimentation profile of DNA (Method I) in CsCl density gradient. (a) DNA from chloroplasts (Method 1a). (b) DNA from chloroplasts by the "laceration technique".

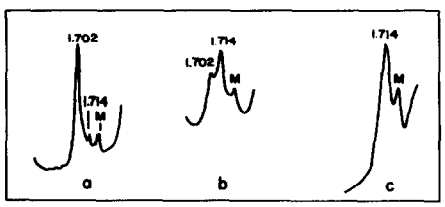

Fig. 4. Densitometer tracings of U/V photographs of DNA density gradient. *M. lysodeikticus* DNA (ρ = 1.731) was used as a marker (M). DNA from (a) whole cells, (b) isolated chloroplasts (Method 1a), (c) chloroplasts isolated by "laceration technique".

(monocotyledons) is that the nuclei are not disrupted and thereby do not contaminate the chloroplasts.

When the DNA of these chloroplasts extracted by Method I was centrifuged to equilibrium in a CsCl gradient only one peak was observed (fig. 3c). This was confirmed by analytical sedimentation which gave one peak with a buoyant density of 1.714 corresponding to chloroplast DNA.

4. Discussion

This investigation has shown that MAK column chromatography is not a satisfactory procedure for separating chloroplast DNA from nuclear DNA in wheat leaves. Moreover the presence of a double peak in the eluate from a DNA sample does not signify the presence of chloroplast DNA as this may be due to partial denaturation.

As far as the authors are aware this is the first characterization of chloroplast DNA in a monocotyledonous plant. Hotta et al. [5] have found a satellite DNA in the cytoplasm of wheat roots with a buoyant density of 1.716 very close to that of wheat chloroplast DNA which may indicate a common origin.

Chloroplasts prepared by a modified procedure of Jensen and Bassham [1] show a DNA profile indicating contamination with nuclear DNA presumably due to fragmentation of nuclei in the Waring blendor. On the other hand the "laceration technique" provides whole chloroplasts free from nuclear contamination. DNA from these preparations sediments in one peak characteristic of chloroplast DNA. Recently Kung and Williams [6] have been successful in preparing chloroplasts from *Vicia faba* free from nuclear contamination using organic solvents and numerous steps. The "laceration technique" is far simpler and produces chloroplasts with an outer membrane.

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